STUDIES ON THE ANTI-STREPTOMYCIN ACTIVITY OF PHOSPHOLIPIDES

BY

LAWRENCE WALLACE BYERS

B. S., Westminster College, 1938

A. M., Oberlin College, 1940

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1948

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R. UNIV. BIBLIOTHEEK LEIDEN

STUDIES ON THE ANTI-STREPTOMYCIN ACTIVITY OF PHOSPHOLIPIDES

Introduction

In their studies on the mode of action of streptomycin, Wallace, Rhymer, Gibson and Shattuck (1) found that brain heart infusion interfered in some way with the action of streptomycin on *Eberthella typhosa* and *Staphylococcus aureus*. They also reported (2) that the bacteriological peptones; Tryptone, Proteose peptone, Peptone, Liver Fraction L, Neopeptone and Phytone, a peptone made from plant proteins, also had an inhibitory effect on the action of streptomycin. It is of especial interest that Phytone was the most active of these materials.

It was the purpose of this investigation to investigate the anti-streptomycin activity of brain heart infusion and of Phytone in order to determine what substance or substances were responsible for this effect.

Discussion Of Results

The anti-streptomycin activity of brain heart infusion was demonstrated by Wallace, *et al.* (1). In attempting to isolate the substance or substances responsible for the anti-streptomycin activity of this material, evidence was obtained indicating that the active factor had some solubility in both organic solvents and water. This behavior suggested that lipositol, an inositol-galactose-containing lipide, might be the active substance.

A sample of soybean lipositol (3) was obtained through the courtesy of Dr. D. W. Woolley. This material proved to have extremely high potency in inhibiting streptomycin (4). However, all attempts to duplicate this preparation from soybean phosphatide failed to yield a substance with comparable

anti-streptomycin activity.

Therefore, attention was turned to brain phosphatide and a systematic fractionation of this material was carried out. Crude brain cephalin was prepared and found to have moderate anti-streptomycin activity, particularly by the *L. arabinosus* assay method. This material was fractionated with organic solvents into inositol phosphatide, phosphatidyl serine and phosphatidyl ethanolamine fractions. There appeared to be no concentration of anti-streptomycin activity in any of these fractions, although the inositol was confined chiefly to the first. Further fractionation of the inositol-containing components of these fractions, however, yielded materials of slightly increased anti-streptomycin activity. In general, these fractions showed higher activity by the *L. arabinosus* assay method than by the *S. aureus* assay method.

Attempts were made to fractionate phosphatides over colums of alumina, magnesia, Superfiltrol, calcium carbonate, magnesium sulfate and sucrose. In general, the materials were either held very tenaciously by the adsorbent or came through in one band.

Since brain heart infusion is a water-soluble material, it was decided to investigate aqueous and aqueous-methanol extracts of brain. Many of these preparations showed high activity, particularly by the *S. aureus* method. It is interesting to note that all of these fractions contained from 5 to 10 per cent of inositol. Whether this inositol is free or bound remains to be investigated. In general, the values for free and total inositol obtained by the yeast assay method were fairly consistant. However, there is the possibility that some bound form of inositol is utilized by the yeast.

Finally, aqueous extracts of brain were prepared and freed of protein by autoclaving at various pH values. These materials showed very potent anti-streptomycin activity, particularly by the *S. aureus* method. It is interesting to note

that when these extracts were dialyzed, the dialysates were very active by the *S. aureus* method but relatively inactive by the *L. arabinosus* method, while the non-dialyzable fractions showed high activity by the latter method.

Summary

A study has been made of the anti-streptomycin activity of soybean phosphatide, of brain phosphatide and of aqueous and aqueous-methanol extracts of brain.

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VITA

The author was born November 12, 1916, in Pulaski Township, Pennsylvania. He received his elementary education in the public schools of that township and attended the high schools of New Castle, Pennsylvania. He entered Westminster College in September, 1934, and received the Bachelor of Science degree from this institution in June, 1938. From September, 1938 to June, 1940, he served as Teaching Assistant and did graduate work in Chemistry at Oberlin College, from which he received the degree of Master of Arts. For the following two years he held the position of Research Assistant in the Rackham Research Foundation at Michigan State College, where he also continued graduate study in Biochemistry.

From July, 1942, to July, 1945, he was a Research Assistant in the Organic Research Laboratory of the Dow Chemical Company. He entered the Graduate School of the University of Illinois in September, 1945, as a Teaching Assistant in Biochemistry, and since January, 1946, has been a Special Research Fellow in the Department of Chemistry.

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